



Warm climate under high CO₂ level in the early Pleistocene based on a leaf fossil assemblage in central Japan

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ABSTRACT

The atmospheric levels of CO₂, a greenhouse gas, are closely related to global change, and investigation of historic CO₂ levels can help achieve a better understanding of the global climate change in the deep past. While stomatal frequency of fossil leaves has been recurrently used for palaeo-CO₂ reconstruction, simultaneous investigation of both palaeo-temperature and palaeo-CO₂ using the same fossil assemblage has been rarely performed. In this study, based on the stomatal analyses of *Quercus gilva* from a leaf bed in the Sayama Formation (1.66–1.55 Ma) in central Japan, we estimated the palaeo-CO₂ values in an interglacial stage (MIS 57 or 55) at 36.41 ± 2.58 pa, which is generally higher than the previously reported data from early Pleistocene. To understand the climate under this high CO₂ level, we also calculated the mean annual temperature (MAT) based on the same leaf assemblage using the leaf margin analysis (LMA) approach. The calculated MAT was 11.0 °C, suggesting a relatively warm climate during the interglacial stage. Our results revealed a warm environment under high CO₂ level during the early Pleistocene, which demonstrates the vital role of CO₂ in controlling the early Pleistocene interglacial temperature.

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1. Introduction

From the latest Pliocene, the climate regime in the Northern Hemisphere changed from warm, ice-free conditions to give rise to an icehouse Earth (Mudelsee and Raymo, 2005; Rohling et al., 2014). The early Pleistocene was characterized by conspicuous glacial–interglacial climate changes on a 41-ka cycle, accompanied by the development of ice sheets in the Northern Hemisphere (Lisiecki and Raymo, 2005). The climatic deterioration during the glacial stages influenced the distribution and composition of terrestrial flora at mid-latitudes in the Northern Hemisphere (van der Hammen et al., 1971; Popescu et al., 2010; Martinetto et al., 2017), and promoted the expansion of boreal vegetation into the lowland areas of central Japan (Momohara, 2016; Yamakawa et al., 2017). However, the warm and oceanic environments during these interglacial stages reappeared in the early Pleistocene, which is apparent from a fossil leaf assemblage of ca. 1.6 Ma in the Sayama Formation on the Pacific side of central Japan (Ito et al., 2017). This warm environment sustained the populations of thermophilous plants and exotic elements that had earlier gone extinct in the other areas of central Japan.

Glacial–interglacial cycles are coupled with atmospheric CO₂ changes, as demonstrated by the Middle and Late Pleistocene ice core records from the Antarctic (Jouzel et al., 2007; Lüthi et al., 2008). As increases in the atmospheric CO₂ are also related to present climate changes (Stocker et al., 2013), palaeo-CO₂ reconstruction of the Pleistocene interglacial stages may provide us a better understanding of present global change. However, palaeo-CO₂ and palaeo-temperature in the early Pleistocene have been chiefly reconstructed for marine environments (Hansen et al., 2008; Hönlisch et al., 2009; Seki et al., 2010; Tripati et al., 2011), and terrestrial records of this period are scarce compared with those in the middle and late Pleistocene, for which ice-core data are available.

Fossil leaves are useful for terrestrial palaeo-temperature and palaeo-CO₂ reconstruction. Morphological characters of fossil leaves are used as climate proxies in the Climate-Leaf Analysis Multivariate Program (CLAMP) (Spicer et al., 2009) and leaf margin analysis (LMA) (Wolfe, 1979; Wilf, 1997; Jacobs, 1999; Gregory-Wodzicki, 2000; Greenwood et al., 2004; Spicer et al., 2004; Traiser et al., 2005; Su et al., 2010). Leaf stomatal frequency has been widely used as a reliable proxy for atmospheric palaeo-CO₂, and has been investigated in different taxa (Kürschner et al., 1996; Wagner et al., 1999; Royer et al., 2001a; Quan et al., 2009; Smith et al., 2010; Doria et al., 2011; Roth-Nebelsick et al., 2012; Sun et al., 2012; Grein et al., 2013; Bai et al., 2015; Hu et al., 2015; Wang et al., 2015). However, simultaneous

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reconstruction of both palaeo-temperature and palaeo-CO₂ using the same fossil assemblage has rarely been carried out.

In this study, we aim to estimate both palaeo-temperature and palaeo-CO₂ based on a leaf fossil assemblage, belonging to an early Pleistocene interglacial stage (MIS 57 or 55), in the Sayama Formation, central Japan (Ito et al., 2017). Based on the stomatal index variation of fossilized Japanese beech (*Fagus crenata*) leaves from the assemblage, Wang et al. (2018) estimated the palaeo-CO₂ difference of the growing environments with altitude, which could reveal the altitudinal transportation of fossilized leaves before their burial. The reconstructed sea-level palaeo-CO₂ based on Japanese beech was estimated at over ca. 36.95–39.60 pa, which is higher than the other published CO₂ data from the early Pleistocene (Wang et al., 2018). To reconstruct the palaeo-CO₂ at sea level and verify our previously reported data, we applied stomatal density (SD) analysis to fossilized leaves of another taxon, *Quercus gilva*, which has the highest temperature requirement among the components of the studied fossil assemblage. In addition, we quantitatively reconstructed the palaeo-temperature based on LMA, to investigate the relationship between palaeo-CO₂ and palaeo-temperature in this interglacial interval.

2. Materials and methods

2.1. Geological setting

The fossil sampling site (35°46'33"N, 139°22'43"E, 130 m a.s.l.; Fig. 1) is an outcrop of the Sayama Formation exposed in the riverbed of the Osawa River in the Sayama Hills, western Tokyo (Saitama Research Group and Kanto Quaternary Research Group, 1970; Nirei and Takegoshi, 2007). The formation is distributed along the western margin of the Kazusa Group sedimentary basin that consists of continuous sedimentation of deep-to-shallow sea deposits in Chiba Prefecture between 2.4 and 0.5 Ma (Ito and Katsura, 1992). The leaf fossil bed is embedded in a 3-m thick fluvial silt overlain by a 7-m thick marine sediment layer. The horizon of the assemblage is 16 m above the Sayama-garasushitu tephra bed (SYG) and 13 m below the Sayama-gomashio tephra bed (SGO) (Ito et al., 2017). The SYG tephra is correlated with the Tsuike Ash, which is situated between the peaks of MIS 57 and 58 in the Uonuma Group in the Niigata Prefecture (Momohara et al.,

2017). The SGO tephra is correlated with the Ob4b-1 tephra that is above the Omn-SK110 tephra (Suzuki et al., 2011), which is correlated with the peak of MIS 54 (Nozaki et al., 2014). Thus, the age of the Sayama fossil leaf bed is constrained to ca. 1.65–1.55 Ma, between MIS 58 and 54 (Sakai and Kurokawa, 2002; Muramatsu, 2008; Suzuki et al., 2011; Momohara et al., 2017).

2.2. Palaeo-pCO₂ reconstruction

As the relationship between SD and palaeo-pCO₂ is species-specific even within a single family (Royer, 2001; Kürschner et al., 2008), it is necessary to select a suitable species among the components of a fossil leaf assemblage as a proxy for sea-level palaeo-pCO₂. Because *Fagus crenata* used in our previous research was distributed along a wide altitudinal range (Wang et al., 2018), we considered using species limited to the lowermost altitude to reconstruct sea-level palaeo-pCO₂. Among the components of the leaf fossil assemblage, *Quercus gilva* has the highest temperature requirements (Ito et al., 2017). *Quercus gilva* is an evergreen broad-leaved tree that is a major component of warm-temperate evergreen broad-leaf forests in southern China (Flora of China Editorial Committee, 1994) and southwestern Japan (Koike, 1989). We considered that the distribution of *Q. gilva* is limited to the lowermost altitude, and that the palaeo-pCO₂ based on its SD reflects sea-level palaeo-pCO₂.

As sun and shade leaves generally exhibit different responses to pCO₂ gradient (Kürschner et al., 1996; Royer et al., 2001b; McElwain, 2004; Kouwenberg et al., 2007), they should be examined separately to establish the correlation between SD and pCO₂. However, the lower and interior position of shade leaves in canopy decreases their chances of wind dispersal to places of fossilization (Kürschner et al., 1996), and their thinner mesophyll and cuticles are susceptible to bacterial degradation (Roth and Dilcher, 1978; Spicer, 1981). Thus, the percentage of shade leaves is generally limited in fossil assemblages (Kürschner, 1997; Hu et al., 2015), and we used only sun leaves in this study.

To distinguish the sun and shade leaves, the epidermal anticlinal wall pattern is considered a useful parameter (Watson, 1942; Kürschner, 1997; Wang et al., 2018), but due to the poor preservation condition of our sample fossil materials, it was not possible to determine the epidermal anticlinal wall pattern. However, most of the *Quercus*

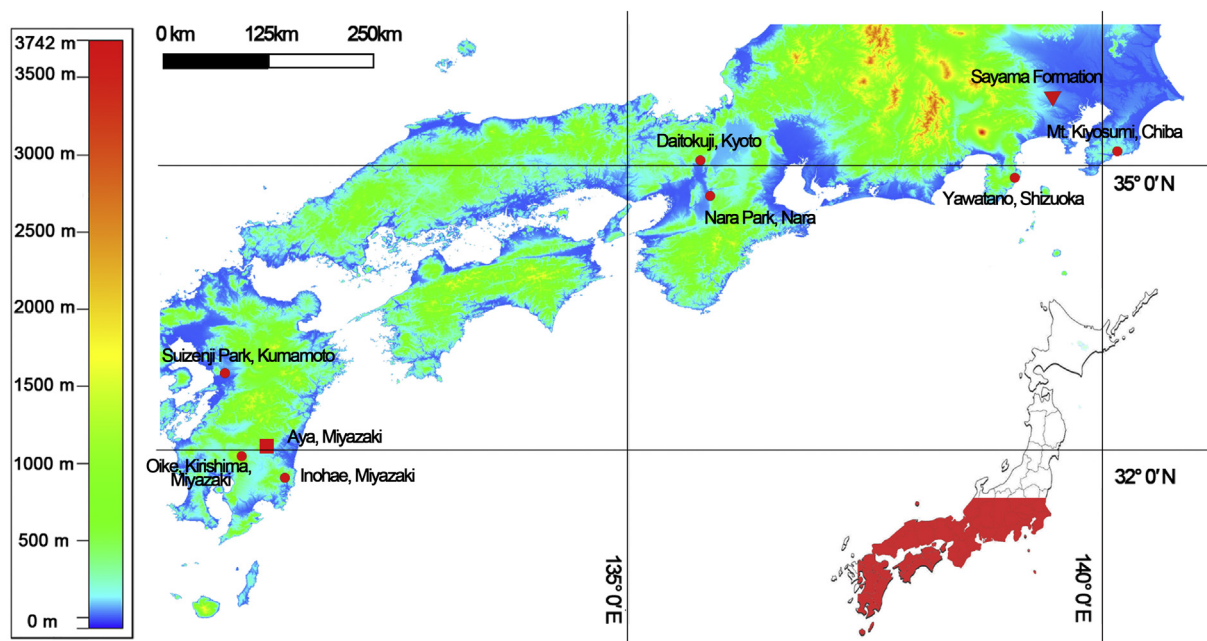


Fig. 1. Map showing the sampling sites for modern leaves (square) and herbarium specimen (square and circle) together with the Sayama Formation (triangle). The original map was downloaded from the Consortium for Spatial Information (CGIAR-CSI; <http://www.cgiar-csi.org/>).

gilva leaves in this fossil assemblage have coriaceous and thick lamina and are believed to be sun leaves, which are distinguishable from the shade leaves, with thin and light-colored lamina.

2.2.1. Stomatal analysis of the extant materials

For palaeo- $p\text{CO}_2$ estimation, we needed to construct a relationship between SD of *Quercus gilva* and atmospheric $p\text{CO}_2$ level, based on modern materials and herbarium specimens that were collected when atmospheric $p\text{CO}_2$ level was lower than that in the present. Sun leaves of *Q. gilva* were collected from five trees each at 200, 400, 518, and 590 m a.s.l. in Aya, the southern part of the Miyazaki Prefecture, south Japan (Fig. 1; Supplementary Data Table S1). All the leaves were collected from the surface of sunny sides of the canopies. Ten sun leaves from each of the five trees were collected for stomatal analysis (Poole, 1999; Beerling and Royer, 2002). In addition, herbarium specimens collected between AD 1961 and 1996, which are stored in the Herbarium of Graduate School of Horticulture, Chiba University (MTDO), were examined. We used five sun leaves from each herbarium specimen for cuticle observation.

For each extant leaf, we first removed the hair on the lower epidermis by washing it in water. Then, we applied a clear nail polish on the lower epidermal surface of the area between two adjacent secondary veins along the mid-vein in the center of the lamina. After air drying the nail polish, we removed it from the leaf to get the cast of the lower epidermal surface. We then enclosed it using glycerin on a glass slide and observed it under a transmitted light microscope connected to a digital camera. Thirty cuticular fields from each tree or each herbarium specimen were photographed for SD calculation.

2.2.2. Calibration curves between stomatal density and $p\text{CO}_2$

We estimated the calibration curves between the SD of *Q. gilva* and $p\text{CO}_2$ based on the extant samples. We calculated the $p\text{CO}_2$ of each sampling spots based on Eq. (1) (Jones, 1992):

$$H = - \ln \left(\frac{p_2}{p_1} \right) \times \frac{R \times T}{MA \times g} \quad (1)$$

p1: $p\text{CO}_2$ (Pa) at sea level

p2: $p\text{CO}_2$ (Pa) at the sampling spot

R: gas constant ($8.3144 \text{ Pa m}^3 \text{ mol}^{-1} \text{ K}^{-1}$)

T: average mean annual temperature (K) between the sampling spot and sea level

MA: molecular weight of air ($0.028964 \text{ kg mol}^{-1}$)

g: acceleration due to gravity (9.8 m s^{-2})

H: elevation (m) of the sampling spot

Accurate T data of each sampling spot were calculated based on National Land Numerical Information (<http://nlftp.mlit.go.jp/ksj/>) for extant materials and “Mesh 2000 Climatic Values in Japan” (JMA, 2011) for herbarium materials, respectively (Supplementary Data Table S1). The data sets are composed of 1 km mesh data of average altitudes and corresponding monthly mean temperatures based on data from ca. 1000 meteorological stations in Japan between 1980–2010 and 1971–2000, respectively. Temperatures at the altitude of the sampling spots were calculated by linear regression of datasets at different altitudes in and around the site. Using these T values along with altitude, past and present sea-level $p\text{CO}_2$ record (p1) (CO_2 Now website, <https://www.co2.earth/>), and other parameters in Eq. (1), $p\text{CO}_2$ (p2) of each spot could be calculated. Next, the calibration curve between stomatal frequencies and $p\text{CO}_2$ for *Quercus gilva* was constructed using SigmaPlot v.12.5.

2.2.3. Palaeo- $p\text{CO}_2$ calculation

For cuticle analysis, we used five fossilized *Quercus gilva* leaves that are stored in the Graduate School of Horticulture, Chiba University (Ito

et al., 2017). We first treated them with 20% HCl for about 6 h to remove calcareous materials, and then washed them until they were neutralized. We enclosed each leaf in glycerin on a glass slide, with the lower epidermis facing up, and observed the same portion that we observed for extant materials under a fluorescence microscope (Nikon Optiphot-2) linked to a digital camera. Five cuticular fields (0.08 mm^2) were photographed for each fossil leaf and the number of stomata in the field was counted to calculate the SD (the number of stomata per mm^2 of leaf). Stomatal index (SI) is not available because of the preservation of the fossilized leaves. Using the SD of our fossil materials in the established correlation, we estimated the sea-level palaeo- CO_2 of the Sayama Formation.

2.3. Palaeo-temperature reconstruction

From the Sayama Formation leaf bed, 39 woody angiosperm species identified by Ito et al. (2017) were used for LMA (Table 1). We classified them into entire- and toothed-margin leaves, and then calculated the percentage of species with entire margin leaves to estimate the MAT. We classified lobed leaves without serration and spinose leaves as entire margin leaves. Different negative correlations between the percentage of entire margin leaf species in woody dicot flora and MAT have been estimated for different regions (Wolfe, 1979; Wilf, 1997; Jacobs, 1999; Gregory-Wodzicki, 2000; Greenwood et al., 2004; Spicer et al., 2004; Traiser et al., 2005; Su et al., 2010), and here, we used the equation for East Asia (Wolfe, 1979) because its sampling area covers the

Table 1

List of dicotyledonous fossil species in the Sayama Formation (Ito et al., 2017).

Family	Fossil species	Score for LMA
Salicaceae	<i>Salix gilgiana</i> Seemen	0
	<i>S. sieboldiana</i> Blume	0
	<i>S. subfragilis</i> Andersson	0
		0
Betulaceae	<i>Alnus</i> cf. <i>japonica</i> (Thunb.) Steud.	0
	<i>Betula grossa</i> Siebold et Zucc.	0
	<i>Carpinus laxiflora</i> (Siebold et Zucc.) Blume	0
	<i>C. tschonoskii</i> Maxim.	0
	<i>Ostrya</i> cf. <i>japonica</i> Sarg.	0
Fagaceae	<i>Fagus crenata</i> Blume	0
	<i>F. japonica</i> Maxim.	0.5
	<i>F. stuxbergi</i> (Nathorst) Tanai	0
	<i>Quercus acutissima</i> Carruth.	0
	<i>Q. serrata</i> Murray	0
	<i>Q. sect. Prinus</i> sp.	0
	<i>Q. gliva</i> Blume	0
	<i>Celtis sinensis</i> Pers.	0
Ulmaceae	<i>Ulmus davidiana</i> Planch. var. <i>japonica</i> (Rehder) Nakai	0
	<i>Zelkova serrata</i> (Thunb.) Makino	0
Lauraceae	<i>Cinnamomum camphora</i> (L.) J. Presl	1
	<i>C. tenuifolium</i> (Makino) Sugim. ex H. Hara	1
	<i>Lindera umbellata</i> Thunb.	1
Cercidiphyllaceae	<i>Cercidiphyllum</i> cf. <i>japonicum</i> Siebold et Zucc. ex Hoffm. et Schult.	0
Hamamelidaceae	<i>Liquidambar formosana</i> Hance	0
Rosaceae	<i>Sorbus</i> cf. <i>commixta</i> Hedl.	0
	<i>Rosa</i> cf. <i>multiflora</i> Thunb.	0
Leguminosae	<i>Albizia kalkora</i> (Roxb.) Prain	1
	<i>Cercis chinensis</i> Bunge	1
	<i>Lepedeza cyrtobotrya</i> Miq.	1
	<i>Wisteria floribunda</i> (Willd.) DC.	1
	Leguminosae (Fabaceae) spp.	1
Aceraceae	<i>Acer diabolicum</i> Blume ex K. Koch	0
	<i>A. palmatum</i> Thunb.	0
	<i>A. pictum</i> Thunb.	1
Hippocastanaceae	<i>Aesculus turbinata</i> Blume	0
Tiliaceae	<i>Tilia</i> sp.	0
Lythraceae	<i>Lagerstroemia indica</i> L.	1
Ericaceae	<i>Ericaceae</i> sp.	1
Oleaceae	<i>Fraxinus japonica</i> Blume ex K. Koch	0
Liliaceae	<i>Smilax china</i> L.	1

entire area of Honshu, Japan. The standard error (SE) of estimated MAT was calculated following Miller et al. (2006).

$$MAT(^{\circ}C) = 1.141 + 30.6 \times p \quad (2)$$

$$SE = b \times \sqrt{[1 + \varphi(n-1)p(1-p)] \times \frac{p(1-p)}{n}} \quad 3$$

p : proportion of woody dicot species with entire leaves

b : slope of the equation; here, 30.6

φ : over dispersion factor, with a value of 0.052 from Miller et al. (2006);

n : total number of woody dicot species among the flora.

3. Results

3.1. Palaeo- pCO_2 reconstruction

Based on the cuticular images of the extant materials (Fig. 2), we calculated their SD data, which showed a significant negative linear relationship ($p < .0001$) with estimated pCO_2 at each of the sampling sites (Fig. 3 and Supplementary Data Table S2).

The average SD value of fossilized *Quercus gilva* leaves was $669.5 \pm 42.1 \text{ mm}^{-2}$ (Supplementary Data Table S3). Plugging this value into the SD- pCO_2 relationship based on extant materials, palaeo- CO_2 was estimated as $36.41 \pm 2.58 \text{ pa}$ ($359 \pm 25 \text{ ppmv}$).

3.2. Palaeo-temperature reconstruction

Among the 39 dicots (Fig. 4 and Table 1), 12 species have entire leaf margin (scored as 1), while the leaves of *Fagus japonica* include both toothed and entire margins (scored as 0.5). Thus, the percentage of species with entire leaf margins (P) was calculated as 32%. The calculated MAT based on the East Asian equation (Eq. (2)) was $11.0^{\circ}C$ and the SE was $\pm 2.67^{\circ}C$ (Eq. (3)).

4. Discussion

4.1. Relationships between stomatal density and pCO_2

The negative correlation of SD values of the extant leaves with pCO_2 (Fig. 3) indicate the sensitivity of the leaves to pCO_2 change. SD is generally believed to be less reliable for palaeo- pCO_2 reconstruction than the SI, which is calculated as the number of stomata divided by the number of epidermal cells. Compared with SI, SD generally exhibits a lower correlation coefficient with pCO_2 (Bai et al., 2015; Hu et al., 2015), possibly owing to the changes induced by leaf expansion, which is susceptible to other environmental factors (Salisbury, 1928; Woodward and Williams, 1987; Kürschner et al., 1996; Kürschner, 1997; Kouwenberg et al., 2007). However, the high correlation coefficient ($r^2 = 0.65$) obtained in this study demonstrates the reliability of using SD in atmospheric pCO_2 reconstruction based on *Quercus gilva* leaves.

Besides *Quercus gilva*, which was used in this study, *Fagus crenata* leaves from the same fossil assemblage have been used to reconstruct palaeo- CO_2 in our previous study (Wang et al., 2018). Based on the SD of *F. crenata*, sea-level palaeo- CO_2 was estimated at more than $36.41 \pm 2.58 \text{ pa}$ ($359 \pm 25 \text{ ppmv}$) (Wang et al., 2018), consistent with the data based on the SD of *Q. gilva* obtained in this study, that is, $36.41 \pm 2.58 \text{ pa}$ ($359 \pm 25 \text{ ppmv}$). However, the sea-level palaeo- CO_2 based on the SI of *F. crenata* was estimated at more than 39.60 pa (391 ppmv) (Wang et al., 2018), higher than the estimated palaeo- CO_2 value obtained in this study.

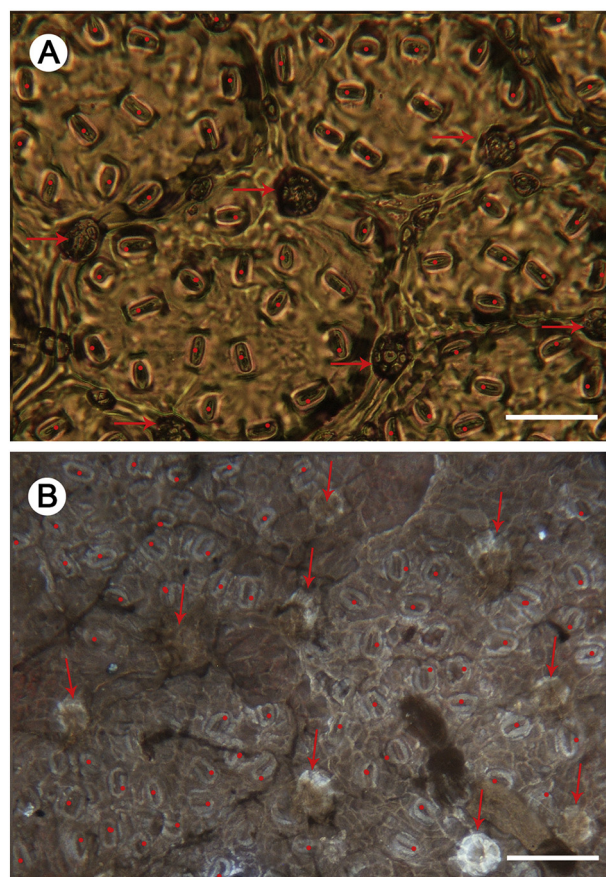


Fig. 2. Cuticular images of modern (A) and fossil (B) *Quercus gilva*. Stomata are marked by red dots, and arrows indicate hair bases. Error bar = 50 μm .

The cross-checking of palaeo- CO_2 data from two species confirms that the sea-level palaeo- CO_2 (over 359 ppmv) in the study area was higher than most of the previous results obtained based on marine materials during the early Pleistocene (Fig. 5). Boron and alkenone data of foraminifera from the Ocean Drilling Program site 999A indicated a CO_2 concentration of around 277 ppmv between 1.5 and 1.65 Ma (Seki et al., 2010). Palaeo- CO_2 data based on B/Ca data (Tripathi et al., 2011) and $\delta^{11}B$ (Hönisch et al., 2009) vary between 200 and 300 ppmv during 1.5–1.7

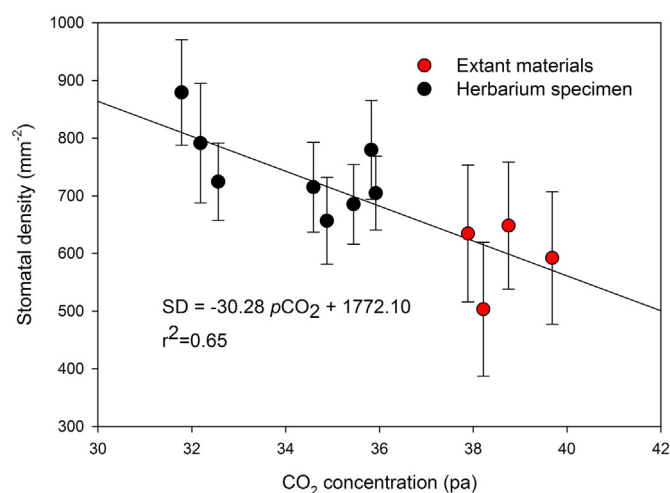


Fig. 3. Relationship between pCO_2 and stomatal density based on the extant sun leaves of *Quercus gilva* (error bar = 1σ).

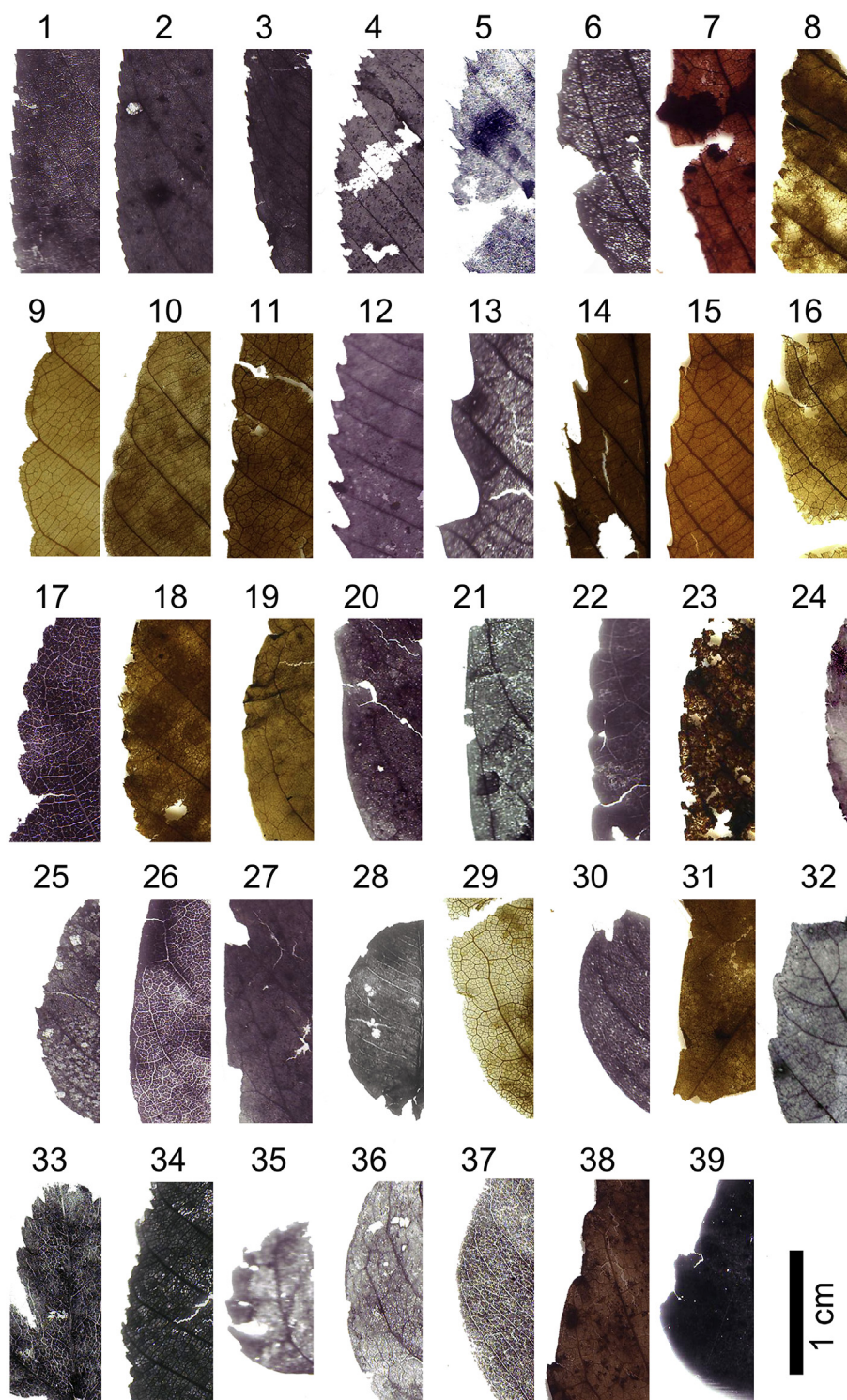


Fig. 4. Leaf margins of fossil leaves for leaf margin analysis (LMA) from the Sayama Formation. 1. *Salix subfragilis* Andersson; 2. *Salix sieboldiana* Blume; 3. *Salix gilgiana* Seemen; 4. *Carpinus laxiflora* (Siebold et Zucc.) Blume; 5. *Carpinus tschonoskii* Maxim.; 6. *Alnus cf. japonica* (Thunb.) Steud.; 7. *Ostrya cf. japonica* Sarg.; 8. *Betula grossa* Siebold et Zucc.; 9. *Fagus crenata* Blume; 10. *Fagus japonica* Maxim.; 11. *Fagus stuxbergi* (Nathorst) Tanai; 12. *Quercus serrata* Murray; 13. *Quercus* sect. *Prinus* sp.; 14. *Quercus acutissima* Carruth.; 15. *Quercus gliva* Blume; 16. *Zelkova serrata* (Thunb.) Makino; 17. *Celtis sinensis* Pers.; 18. *Ulmus davidiana* Planch. var. *japonica* (Rehder) Nakai; 19. *Lindera umbellata* Thunb.; 20. *Cinnamomum camphora* (L.) J. Presl; 21. *Cinnamomum tenuifolium* (Makino) Sugim. ex H. Hara; 22. *Cercidiphyllum cf. japonicum* Siebold et Zucc. ex Hoffm. et Schult.; 23. *Liquidambar formosana* Hance; 24. *Sorbus cf. commixta* Hedl.; 25. *Rosa cf. multiflora* Thunb.; 26. *Albizia kalkora* (Roxb.) Prain; 27. *Wisteria floribunda* (Willd.) DC.; 28. *Lespedeza cyrtobotrya* Miq.; 29. *Cercis chinensis* Bunge; 30. Leguminosae (Fabaceae) spp.; 31. *Acer pictum* Thunb.; 32. *Acer diabolicum* Blume ex K. Koch; 33. *Acer palmatum* Thunb.; 34. *Aesculus turbinata* Blume; 35. *Tilia* sp.; 36. *Lagerstroemia indica* L.; 37. Ericaceae sp.; 38. *Fraxinus japonica* Blume ex K. Koch; 39. *Smilax china* L.

Ma. A similar high CO₂ level of ca. 360 ppmv was recorded by B/Ca (Tripati et al., 2011) at around 1.44 Ma: an age that is a little younger than the age of our data. Although the Pleistocene CO₂ results derived from different proxies are relatively consistent with each other

compared with the records in the Neogene, most of the results still vary from ca. 190 to 360 ppmv (Beerling and Royer, 2011).

Compared with the published CO₂ data based on stomatal method applied to the last 5 Ma (van der Burgh et al., 1993; Kürschner et al.,

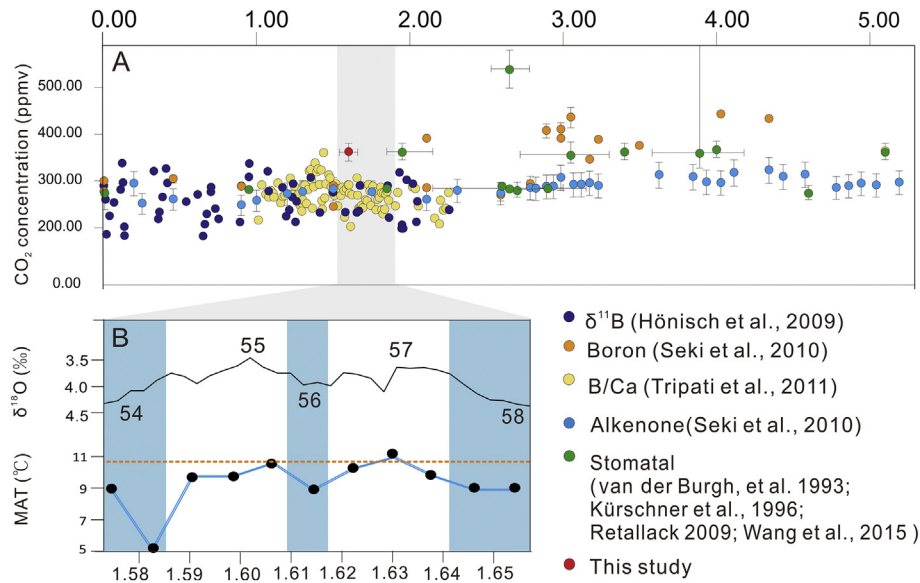


Fig. 5. (A) Palaeo-CO₂ data derived from different proxies since 5 Ma. (B) Mean annual temperature (MAT) curve based on the early Pleistocene Uonuma Group (Momohara et al., 2017) compared with the $\delta^{18}\text{O}$ record (Lisiecki and Raymo, 2005). The orange broken line indicates the palaeo-temperature reconstructed from the Sayama leaf bed.

1996; Retallack, 2009; Wang et al., 2015), we can see a stable CO₂ level at around 350 ppmv between 3 and 4 Ma, corresponding to the middle Pliocene warming (Dowsett et al., 1996; Haywood et al., 2016). Subsequently, decreased palaeo-CO₂ levels of around 300 ppmv were recorded for the latest Pliocene cooling phase between 2.8 and 2.5 Ma, except for some extraordinary data showing more than 500 ppmv at 2.6 Ma (Bai et al., 2015), which may be affected by the abnormal emission of CO₂ from wetlands. In addition, high-resolution CO₂ data based on ice core analysis indicates a noticeable difference in CO₂ level between glacial and interglacial stages during the Pliocene: 180 ppmv in the glacials and 280–300 ppmv in the interglacials (Jouzel et al., 2007; Lüthi et al., 2008). Early Pleistocene CO₂ levels varied between ca. 280 and 360 ppmv, and a record of ca. 360 ppmv in 1.9 Ma (Kürschner et al., 1996) indicates a similar high CO₂ level to our data.

4.2. Relationships between palaeo-pCO₂ and palaeotemperature

Consistent with this relatively high CO₂ level recorded, the palaeo-temperature reconstructed on the basis of LMA was also high. Momohara et al. (2017) reconstructed the palaeotemperature for the same time based on the coldest limiting temperature of thermophilous taxon using plant megafossil assemblages of the Uonuma Group, central Japan (Fig. 4, B). The MAT of interglacials peak in MIS 57 (11.0 °C) and MIS 55 (10.6 °C) (Momohara et al., 2017), which is almost equivalent to our contemporaneous palaeo-temperature data. The assemblage composition of the Sayama leaf bed is characterized by evergreen broad-leaved trees including *Actinodaphne*, *Cinnamomum*, and *Quercus* subgen. *Cyclobalanopsis* (Ito et al., 2017). Our former stomatal study of fossilized *F. crenata* leaves from the same assemblage clarified that altitudinal transportation of leaves before burial was greater than 1000 m (Wang et al., 2018). This altitudinal transportation caused a mixture of different climate elements in the Sayama Formation: cool-temperate elements such as *Betula glossa*, *Cercidiphyllum japonicum*, and *Aesculus turbidata*; warm-temperate elements such as *Ginkgo biloba*, *Cercis chinensis*, *Liquidambar formosana*, and *Cunninghamia lanceolata* var. *konishii*; and subtropical elements such as *Quercus gilva*, *Cinnamomum yabunikei*, *Albizia kalkora*, and *Lagerstroemia indica* (Ito et al., 2017). Because cool temperate elements from higher altitude tend to be serrated, LMA based on a leaf assemblage that includes high altitude plants will produce a cooler

MAT result. Based on our palaeo-temperature and palaeo-CO₂ results, the fossil assemblage undoubtedly indicates a warm, interglacial environment, which is also confirmed by the position of the leaf bed just below the marine transgressive horizon (Ito et al., 2017). Thus, our results indicate a warm climate under high pCO₂ level during an interglacial stage of the early Pleistocene.

Many studies have documented a coupled relationship between CO₂ and temperature during the Pleistocene. Atmospheric CO₂ level change based on fossil *Metasequoia* needles from Japan exhibits a similar decreasing trend with palaeo-temperature since the late Miocene to early Pleistocene (Wang et al., 2015). Research on Antarctic ice cores has also demonstrated a positive relationship between palaeo-CO₂ and palaeo-temperature (Jouzel et al., 2007; Lüthi et al., 2008) during the Middle and Late Pleistocene. Evidence from other geological times also show that atmospheric CO₂ levels have played an important role in global climate change (Petit et al., 1999; Bijl et al., 2010; Pagani et al., 2011). Our research provides evidence to support the coupling of high palaeo-CO₂ and high palaeo-temperature episodes during the early Pleistocene, based on a terrestrial plant assemblage. However, it is important to carry out stomatal studies together with palaeo-temperature reconstruction for other interglacial stages as well, which could help to reveal the role of atmospheric CO₂ in relation to global temperature changes in the early Pleistocene.

5. Conclusions

In this study, we reconstructed the palaeo-CO₂ and palaeo-temperature during an interglacial stage in MIS 57 or 55 (1.66–1.55 Ma), based on a leaf bed in the Sayama Formation, central Japan. According to the SD exhibited by leaves of *Q. gilva*, the palaeo-CO₂ was estimated to have been 36.41 ± 2.58 pa, which is at a higher level than previously reported. Based on the LMA of woody dicots in the fossil assemblage, the palaeo-temperature was estimated at 11.0 °C, which is almost equivalent to the contemporaneous data from the Uonuma Group, central Japan. Our results indicate a warm climate under high pCO₂ level in an interglacial stage of the early Pleistocene, demonstrating the vital role of CO₂ in controlling the early Pleistocene interglacial temperature.

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